Association of Tumor Mutational Burden and PD-L1 with the Efficacy of Pembrolizumab with or without Chemotherapy versus Chemotherapy in Advanced Urothelial Carcinoma

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ABSTRACT

Purpose: The three-arm, phase III KEYNOTE-361 study did not meet its dual primary endpoints of progression-free survival (PFS) or overall survival (OS) with first-line pembrolizumab plus chemotherapy versus chemotherapy in advanced urothelial carcinoma. This prespecified exploratory analysis assessed the association of tumor mutational burden (TMB) and PD-L1 combined positive score (CPS) with clinical outcomes.

Patients and Methods: TMB and PD-L1 CPS were determined via whole-exome sequencing and PD-L1 IHC 22C3 pharmDx, respectively. The association was evaluated in each treatment arm using logistic regression [objective response rate (ORR)] and Cox proportional hazards regression models (PFS and OS); one-sided (pembrolizumab monotherapy; pembrolizumab plus chemotherapy) and two-sided (chemotherapy) nominal P values were calculated. Significance was prespecified at $\alpha = 0.05$ without multiplicity adjustment. Efficacy was evaluated by prespecified cutoffs of 175 mutations/exome (TMB) and CPS 10 (PD-L1).

Results: Of the 993 treated patients, 820 (82.6%) and 993 (100%) had evaluable TMB and CPS data, respectively. Continuous TMB was positively associated with ORR, PFS, and OS for pembrolizumab monotherapy (one-sided $P < 0.001$, $P < 0.001$, and $P = 0.007$, respectively); PFS and OS for pembrolizumab plus chemotherapy (one-sided $P = 0.007$ and $P = 0.010$, respectively); and OS for chemotherapy alone (two-sided $P = 0.040$). Continuous PD-L1 CPS showed evidence of anticipated association with ORR and PFS for pembrolizumab monotherapy. The subgroup with TMB ≥175 mutations/exome and PD-L1 CPS ≥10 had the highest PFS and OS improvements with pembrolizumab alone or with chemotherapy versus chemotherapy alone.

Conclusions: These data suggest that TMB may be predictive of the response to pembrolizumab alone or with chemotherapy in advanced urothelial carcinoma.

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Translational Relevance

This prespecified exploratory analysis from the phase III KEYNOTE-361 study was conducted to evaluate the association of tumor mutational burden (TMB) and PD-L1 combined positive score with clinical outcomes of pembrolizumab monotherapy, pembrolizumab plus chemotherapy, or chemotherapy alone in advanced urothelial carcinoma. TMB was associated with outcomes for pembrolizumab and, to a lesser extent, for pembrolizumab plus chemotherapy in advanced urothelial carcinoma. Survival benefit of pembrolizumab alone or with chemotherapy versus chemotherapy was highest for the subgroup with TMB ≥175 mutations/exome and PD-L1 combined positive score ≥10. These data support the clinical utility of TMB in identifying patients with advanced urothelial carcinoma who are likely to respond to pembrolizumab alone or with chemotherapy. Considering the new and emerging first-line immunotherapy-based treatments for advanced urothelial carcinoma, the role of these biomarkers in determining response in this disease setting warrants continued evaluation.

Introduction

Urothelial carcinoma accounts for approximately 90% of all bladder cancers (1). Immunotherapy with or without other therapies is the mainstay treatment for patients with advanced or metastatic urothelial carcinoma (2–4). Given that the efficacy of treatment may depend on patient tumor characteristics (5, 6), discovering clinically relevant and highly reliable prognostic and/or predictive biomarkers may help identify patients with advanced urothelial carcinoma who will respond effectively to immunotherapy-based treatments. Several such biomarkers, including tumor PD-L1 expression, have been identified for PD-(L)1 inhibitors across several solid tumor types (7, 8); however, exploratory analyses evaluating PD-L1 status and outcomes in patients with advanced or metastatic urothelial carcinoma have yielded conflicting results in the postplatinum and platinum-eligible/cisplatin-ineligible settings (9, 10). In particular, the potential of PD-L1 combined positive score (CPS) as a biomarker predictive of response to pembrolizumab monotherapy or pembrolizumab-based combination therapies in advanced or metastatic urothelial carcinoma is currently unclear.

In recent years, tumor mutational burden (TMB) has emerged as a biomarker predictive of response to immunotherapies in the pan-tumor setting (11–14). Associations between TMB and response to pembrolizumab in advanced or metastatic urothelial carcinoma were shown in exploratory studies from the single-arm, phase II KEYNOTE-052 study of first-line pembrolizumab monotherapy in cisplatin-ineligible patients with advanced or metastatic urothelial carcinoma and the randomized phase III KEYNOTE-045 study of pembrolizumab versus chemotherapy in patients with previously treated advanced urothelial carcinoma (9).

In the three-arm, open-label, phase III KEYNOTE-361 study, first-line pembrolizumab with or without platinum-based chemotherapy versus chemotherapy alone was evaluated in patients with advanced urothelial carcinoma regardless of tumor PD-L1 status (15). The study did not meet its primary endpoints of superior progression-free survival [PFS; HR, 0.78; 95% confidence interval (CI), 0.65–0.93; $P = 0.0033$] or overall survival (OS; HR, 0.86; 95% CI, 0.72–1.02; $P = 0.0407$) for pembrolizumab plus chemotherapy versus chemotherapy (15). Exploratory analysis of pembrolizumab monotherapy versus chemotherapy showed similar OS (HR, 0.92; 95% CI, 0.77–1.11).

We explored the association of TMB and PD-L1 expression with clinical outcomes of pembrolizumab alone and pembrolizumab plus chemotherapy compared with chemotherapy alone in patients with advanced urothelial carcinoma from the KEYNOTE-361 study.

Patients and Methods

Study design, participants, and treatment

KEYNOTE-361 (ClinicalTrials.gov, NCT02853305) was a randomized, open-label, phase III study conducted across 201 medical centers globally (15). Details of the study design and the eligibility criteria have been reported (15). Briefly, key eligibility criteria included patients ≥18 years of age with histologically or cytologically confirmed, locally advanced, unresectable, or metastatic urothelial carcinoma of the renal pelvis, bladder, ureter, or urethra; an Eastern Cooperative Oncology Group performance status (ECOG PS) score between 0 and 2; and no previous systemic anticancer treatment. Eligible patients had ≥1 measurable lesion as per RECIST v1.1 and provided either a newly obtained or an archival tumor sample for PD-L1 analysis.

Patients were randomly assigned in a 1:1:1 ratio to receive pembrolizumab monotherapy, pembrolizumab plus platinum-based chemotherapy (gemcitabine plus investigator's choice of either cisplatin or carboplatin), or platinum-based chemotherapy. After the randomization of approximately 82% of the total patients enrolled, on the basis of emerging survival data for PD-(L)1 inhibitor monotherapy in patients with a PD-L1 CPS of <10 in the KEYNOTE-361 and IMvigor130 (16) studies, the protocol was amended to limit randomization of patients with a PD-L1 CPS of <10 to only the pembrolizumab plus chemotherapy or chemotherapy-alone arm (15).

The study protocol and all amendments were approved by each participating institution's institutional review board or ethics committee. The study was conducted in accordance with the protocol, its amendments, the ethical principles originating from the Declaration of Helsinki, and Good Clinical Practice guidelines. All patients provided written informed consent before enrollment.

Outcomes and assessment

The primary objective of this exploratory analysis was to determine whether TMB and PD-L1 CPS as continuous variables were associated with clinical outcomes [objective response rate (ORR), PFS, and OS] in the three treatment arms. The secondary objectives were to evaluate the clinical outcomes for pembrolizumab monotherapy versus chemotherapy or pembrolizumab plus chemotherapy versus chemotherapy in subgroups defined using prespecified cutoffs for TMB and PD-L1 CPS.

TMB was determined via whole-exome sequencing (WES) using formalin-fixed, paraffin-embedded pretreatment tumor samples and matched DNA, as previously described (11, 13). Briefly, after pathology assessment, tissue was scraped from the entire section using a fresh scalpel and transferred to a 1.5-mL tube containing 200 μL of 100% ethanol. DNA was isolated using the QIAamp DNA FFPE Tissue Kit (Qiagen). Thereafter, the tumor DNA was quantitated using the Qubit assay (Invitrogen), and the quality was assessed

using the Quantidex qPCR DNA QC Assay (Asuragen). Matched normal DNA was extracted from whole blood collected in a PAXgene Blood DNA Tube (Qiagen) at clinical sites and stored at −20°C or −70°C/80°C until processed in an approved central laboratory identified by the sponsor. The Chemagic STAR DNA Blood Kit (PerkinElmer) run on either a Hamilton Chemagic STAR or PerkinElmer Chemagic 360 automated instrument was used to extract DNA in a final volume of 500 μL or 1.0 mL. Extracted DNA was subjected to volume and concentration determination and ultraviolet and visible spectral analysis to assess purity. WES was performed using the ACE Cancer Exome (Personalis).

The WES bioinformatics pipeline was implemented as previously described (11) and included the alignment of WES reads to the Genome Reference Consortium Human Build 37 (RRID: SCR_006553) using Burrows–Wheeler Aligner MEM (V.0.7.12, RRID: SCR_010910) followed by preprocessing steps such as duplicate marking, indel realignment, and base recalibration with Picard (V.1.114, RRID: SCR_006525) and Genome Analysis Toolkit (V.2, RRID: SCR_006390) to generate an analysis-ready binary alignment map. TMB was defined as the number of somatic nonsynonymous single-nucleotide variants and indels that met the predetermined criteria (11, 13). An OncoPrint showing biomarker distribution and the most frequently mutated genes was generated using the ComplexHeatmap (version 2.6.2, RRID: SCR_017270) package of R (version 4.0.5, RRID: SCR_001905). Mutations were processed from WES data using the TMB pipeline. Apolipoprotein B mRNA-editing enzyme, catalytic polypeptides (APOBECs) were determined from WES data based on the mutation signature score method (17) after adjusting for TMB.

PD-L1 expression was determined using PD-L1 IHC 22C3 pharmDx (Agilent Technologies, RRID: AB_2889976). PD-L1 CPS was calculated as the number of PD-L1–staining cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells, multiplied by 100. PD-L1 positivity was predefined as CPS ≥10.

ORR was defined as the proportion of patients with a confirmed complete response or partial response per RECIST v1.1 by blinded independent central review. PFS was defined as the time from randomization to the first radiographically confirmed disease progression per RECIST v1.1 by blinded independent central review or death from any cause (whichever occurred first). OS was defined as the time from randomization to death from any cause. Tumor response was assessed by radiographic tumor imaging using CT or MRI every 9 weeks for the first 54 weeks and every 12 weeks thereafter.

Statistical analysis

This prespecified exploratory analysis included all treated patients in KEYNOTE-361 with available TMB and/or PD-L1 CPS data that passed quality control. Analyses followed a statistical analysis plan written before merging clinical data with biomarker assessment, specifying where statistical testing would be used and what TMB and PD-L1 CPS cutoffs defined the subgroups for treatment arm comparisons.

The associations between continuous TMB and PD-L1 CPS and clinical outcomes were evaluated using the logistic regression model for ORR and the Cox proportional hazards regression model for PFS and OS, adjusting for ECOG PS. Nominal *P* values were calculated using a one-sided Wald test for pembrolizumab monotherapy and pembrolizumab plus chemotherapy (positive association hypothesized) and a two-sided Wald test for chemotherapy alone (no assumed direction hypothesized). Significance was prespecified at $\alpha = 0.05$ without multiplicity adjustment. The Spearman correlation between TMB and PD-L1 CPS was calculated, and the joint pattern of TMB and PD-L1 CPS with objective response was plotted for each treatment arm in patients with available TMB and PD-L1 CPS data.

To evaluate the impact of TMB and PD-L1 CPS on comparative efficacy, patients were categorized into two subgroups per predefined cutoffs of ≥175 mutations/exome and <175 mutations/exome for TMB (175 mutations/exome via WES is equivalent to 10 mutations/megabase using FoundationOne CDx; refs. 11, 18) and CPS ≥10 and CPS <10 for PD-L1. The TMB cutoff was based on TMB-high threshold of ≥175 mutations/exome previously verified to enrich for response to pembrolizumab in the pan-tumor setting, correlated with 10 mutations/megabase using FoundationOne CDx and associated with peak statistical significance for differences in inflammation in the tumor microenvironment as measured by the 18-gene T-cell–inflamed gene expression profile (11, 13). PFS and OS were estimated using the Kaplan–Meier method. The Cox proportional hazards regression model fit within the subgroup was used to estimate the HR and 95% CI between treatments with adjustment for ECOG PS. The clinical data cutoff date for this analysis was April 29, 2020.

Data availability

Merck Sharp & Dohme LLC (MSD), a subsidiary of Merck & Co., Inc., Rahway, NJ, is committed to providing qualified scientific researchers access to anonymized data and clinical study reports from the company's clinical trials for the purpose of conducting legitimate scientific research. MSD is also obligated to protect the rights and privacy of trial participants and, as such, has a procedure in place for evaluating and fulfilling requests for sharing company clinical trial data with qualified external scientific researchers. The MSD data-sharing website (available at: http://engagezone.msd.com/ds_documentation.php) outlines the process and requirements for submitting a data request. Applications will be promptly assessed for completeness and policy compliance. Feasible requests will be reviewed by a committee of MSD subject matter experts to assess the scientific validity of the request and the qualifications of the requestors. In line with data privacy legislation, submitters of approved requests must enter into a standard data-sharing agreement with MSD before data access is granted. Data will be made available for requests after product approval in the United States and the European Union or after the product development is discontinued. There are circumstances that may prevent MSD from sharing requested data, including country- or region-specific regulations. If the request is declined, it will be communicated to the investigator. Access to genetic or exploratory biomarker data requires a detailed, hypothesis-driven statistical analysis plan collaboratively developed by the requestor and MSD subject matter experts. After approval of the statistical analysis plan and execution of a data-sharing agreement, MSD will either perform the proposed analyses and share the results with the requestor or construct biomarker covariates and add them to a file with clinical data that is uploaded to an analysis portal so that the requestor can perform the proposed analyses.

Results

Patients

Between October 19, 2016, and June 29, 2018, 1,010 patients were randomly assigned to receive pembrolizumab monotherapy, pembrolizumab plus chemotherapy, or chemotherapy. The median follow-up, defined as the time from randomization to data cutoff (April 29, 2020), in the total KEYNOTE-361 population was 31.7 months (IQR, 27.7–36.0). Of the 993 patients who received ≥1

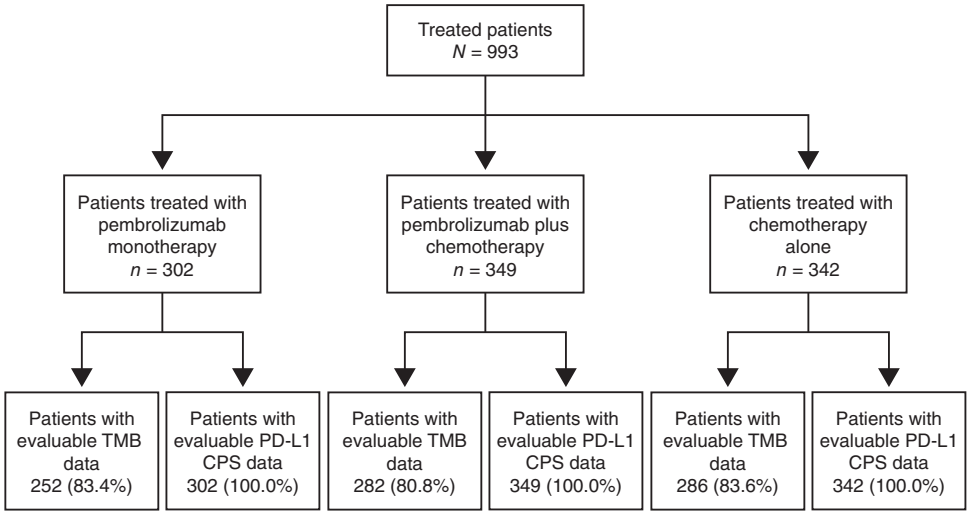

Figure 1.
Evaluable data profile.

dose of study treatment, TMB data were evaluable for 820 patients (82.6%; pembrolizumab monotherapy, 252; pembrolizumab plus chemotherapy, 282; and chemotherapy, 286). All 993 treated patients (100%; pembrolizumab, 302; pembrolizumab plus chemotherapy, 349; and chemotherapy, 342) had evaluable PD-L1 CPS data (**Fig. 1**). Most patients had tumors with TMB <175 mutations/exome and PD-L1 CPS <10; *TP53* was the most commonly mutated gene (52%; Supplementary Fig. S1). Baseline characteristics in the evaluable TMB population were comparable with the intention-to-treat study population ($N = 993$; as well as the evaluable PD-L1 CPS population) and were generally well balanced between treatment arms (Supplementary Tables S1 and S2; ref. 15).

Association between TMB and PD-L1 CPS and clinical outcomes

TMB as a continuous variable was significantly associated with ORR, PFS, and OS for pembrolizumab monotherapy (one-sided $P < 0.001$, $P < 0.001$, and $P = 0.007$, respectively). It was associated with only PFS and OS for pembrolizumab plus chemotherapy (one-sided $P = 0.007$ and $P = 0.010$, respectively) and with only OS for chemotherapy (two-sided $P = 0.040$; **Table 1**). TMB was higher in responders than in nonresponders treated with pembrolizumab monotherapy but did not differ between responders and nonresponders treated with pembrolizumab plus chemotherapy or with chemotherapy alone (**Fig. 2A**). The area under the ROC curve (AUROC) for discriminating TMB as a predictor of objective response was 0.64 (95% CI, 0.56–0.71) for pembrolizumab monotherapy, 0.53 (95% CI, 0.46–0.60) for pembrolizumab plus chemotherapy, and 0.52 (95% CI, 0.45–0.59) for chemotherapy (**Fig. 2B** and **C**).

Although trends in the anticipated direction were observed for both ORR and PFS in the pembrolizumab monotherapy and pembrolizumab plus chemotherapy arms, PD-L1 CPS as a continuous variable only met the nominal significance threshold for pembrolizumab monotherapy with PFS (one-sided $P = 0.006$) and with ORR for pembrolizumab plus chemotherapy (one-sided $P = 0.042$; **Table 1**). PD-L1 CPS as a continuous variable was not significantly

Table 1. Association of nominal *P* values between TMB and PD-L1 CPS as continuous variables and clinical outcomes by treatment arm using a univariate model.

	TMB (log_{10})			PD-L1 CPS		
Outcome	**Pembrolizumab[a] (*n* = 252)**	**Pembrolizumab + chemotherapy[a] (*n* = 282)**	**Chemotherapy[b] (*n* = 286)**	**Pembrolizumab[a] (*n* = 302)**	**Pembrolizumab + chemotherapy[a] (*n* = 349)**	**Chemotherapy[b] (*n* = 342)**
ORR per RECIST v1.1 by BICR	**<0.001**	0.324	0.924	0.056	**0.042**	0.536
PFS per RECIST v1.1 by BICR	**<0.001**	**0.007**	0.368	**0.006**	0.066	0.901
OS	**0.007**	**0.010**	**0.040**[c]	0.472	0.054	0.442

Abbreviation: BICR, blinded independent central review.
The association was evaluated using logistic regression (ORR) and Cox proportional hazards regression (PFS and OS), with adjustment for ECOG PS. One-sided (pembrolizumab monotherapy and pembrolizumab plus chemotherapy arms) and two-sided (chemotherapy arm) nominal *P* values were calculated. Significance was prespecified at $\alpha = 0.05$. Bold indicates significance.
[a]Positive association hypothesized.
[b]No assumed direction hypothesized.
[c]Positive association observed.

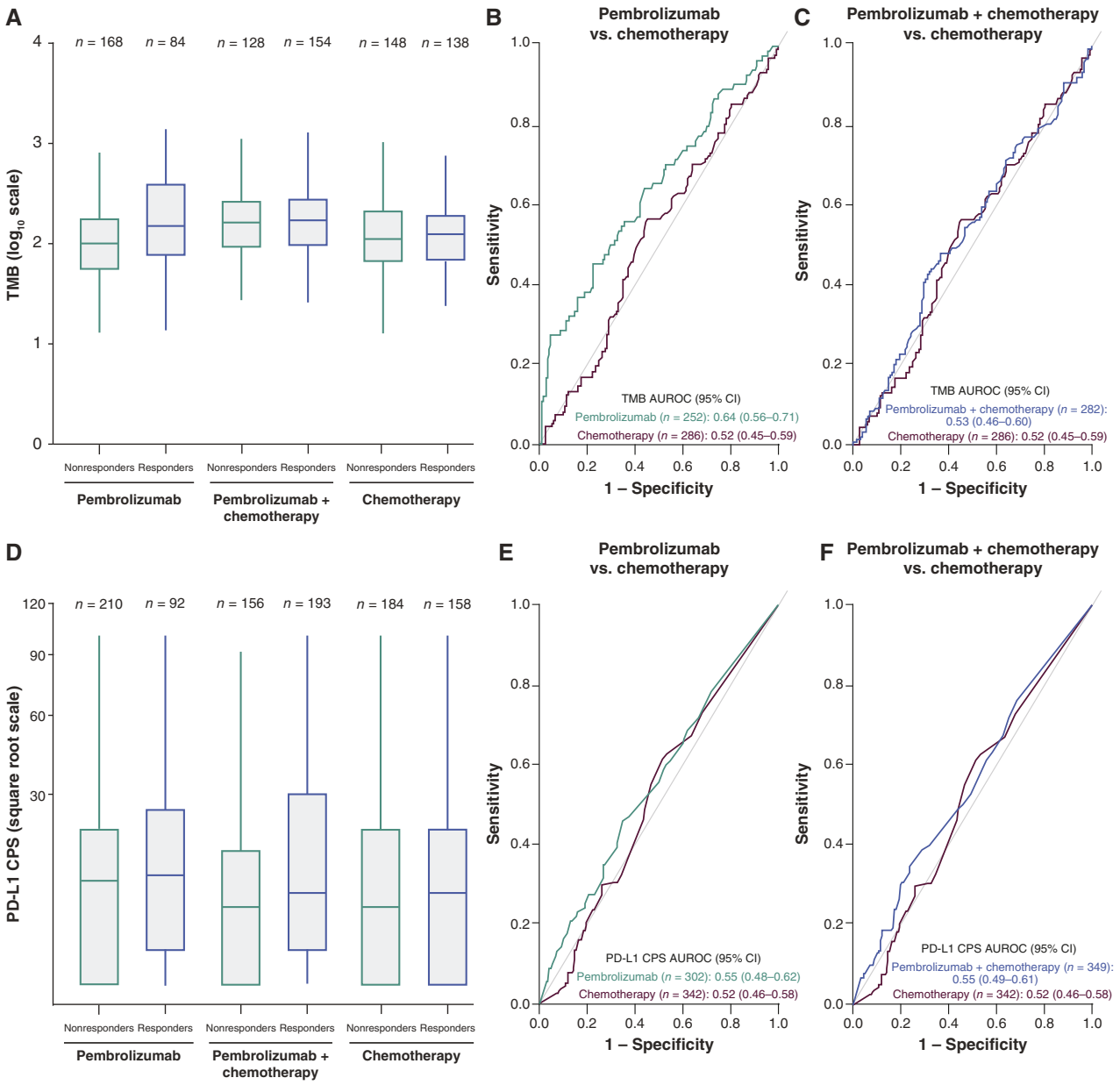

Figure 2.

Patient-level biomarker score distribution by response status and treatment arm and AUROC for biomarkers as predictors of objective response. **A–C,** TMB. **D–F,** PD-L1 CPS.

associated with clinical outcomes for chemotherapy. A shift toward higher PD-L1 CPS distributions for responders versus nonresponders was observed for the pembrolizumab monotherapy and the pembrolizumab plus chemotherapy arms (**Fig. 2D**). The AUROC for discriminating PD-L1 CPS as a predictor of objective response was 0.55 (95% CI, 0.48–0.62) for pembrolizumab monotherapy, 0.55 (95% CI, 0.49–0.61) for pembrolizumab plus chemotherapy, and 0.52 (95% CI, 0.46–0.58) for chemotherapy (**Fig. 2E** and **F**).

The Spearman ρ for the correlation between pretreatment TMB and PD-L1 CPS was modest in all three treatment arms (0.22 for pembrolizumab monotherapy, 0.15 for pembrolizumab plus chemotherapy, and 0.21 for chemotherapy).

Efficacy estimates by TMB and PD-L1 CPS cutoffs

When evaluating ORR for pembrolizumab monotherapy according to subgroup defined by TMB and PD-L1 CPS cutoffs, ORR was notably higher in the subgroup with both TMB ≥175 mutations/exome and PD-L1 CPS ≥10 than other dual biomarker subgroups, a pattern not observed for pembrolizumab plus chemotherapy or chemotherapy (Supplementary Fig. S2). At the

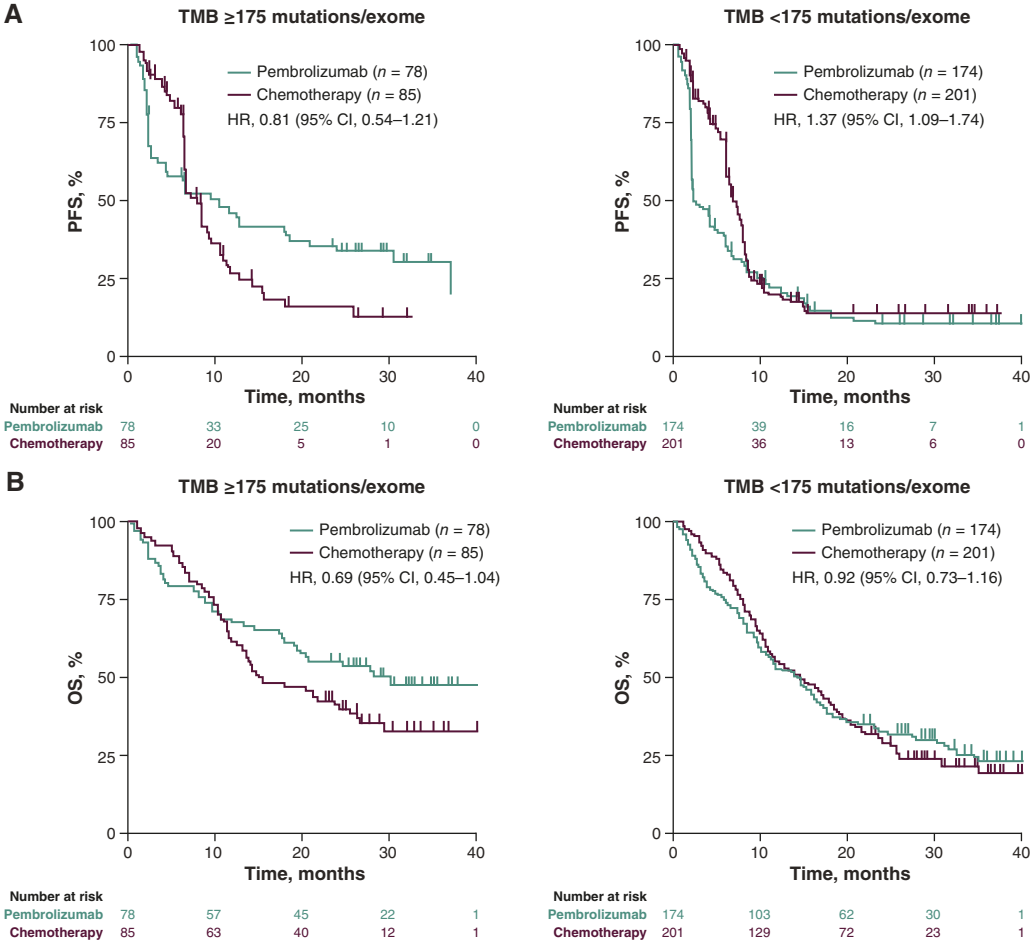

Figure 3.
Kaplan–Meier estimates of survival by TMB cutoff for pembrolizumab monotherapy vs. chemotherapy. **A,** PFS. **B,** OS. NE, not estimable.

prespecified TMB-high cutoff of 175 mutations/exome, with pembrolizumab monotherapy versus chemotherapy, the median PFS was 9.9 months versus 7.8 months (HR, 0.81; 95% CI, 0.54–1.21) in the TMB ≥175 mutations/exome subgroup and 2.3 months versus 7.1 months (HR, 1.37; 95% CI, 1.09–1.74) in the TMB <175 mutations/exome subgroup (**Fig. 3A**). The median OS was 28.3 months versus 15.5 months (HR, 0.69; 95% CI, 0.45–1.04) in the TMB ≥175 mutations/exome subgroup and 14.3 months versus 14.4 months (HR, 0.92; 95% CI, 0.73–1.16) in the TMB <175 mutations/exome subgroup (**Fig. 3B**). For pembrolizumab plus chemotherapy versus chemotherapy, the median PFS was 10.3 months versus 7.8 months (HR, 0.70; 95% CI, 0.48–1.02) in the TMB ≥175 mutations/exome subgroup and 8.1 months versus 7.1 months (HR, 0.80; 95% CI, 0.63–1.00) in the TMB <175 mutations/exome subgroup (**Fig. 4A**), whereas median OS was 24.4 months versus 15.5 months (HR, 0.77; 95% CI, 0.53–1.14) in the TMB ≥175 mutations/exome subgroup and 15.9 months versus 14.4 months (HR, 0.79; 95% CI, 0.63–0.99) in the TMB <175 mutations/exome subgroup (**Fig. 4B**).

At the prespecified PD-L1 CPS 10 cutoff, with pembrolizumab versus chemotherapy, the median PFS was 4.0 months versus 7.4 months (HR, 1.32; 95% CI, 1.01–1.74) in the PD-L1 CPS ≥10 subgroup and 2.3 months versus 6.7 months (HR, 1.28; 95% CI, 1.00–1.65) in the PD-L1 CPS <10 subgroup (**Fig. 5A**). The median OS was 16.0 months versus 16.9 months (HR, 1.03; 95% CI, 0.78–1.35) in the PD-L1 CPS ≥10 subgroup and 13.7 months versus 14.0 months (HR, 0.83; 95% CI, 0.64–1.07) in the PD-L1 CPS <10 subgroup (**Fig. 5B**). For pembrolizumab plus chemotherapy versus chemotherapy, the median PFS was 8.5 months versus 7.4 months (HR, 0.76; 95% CI, 0.58–1.01) in the PD-L1 CPS ≥10 subgroup and 8.3 months versus 6.7 months (HR, 0.76; 95% CI, 0.60–0.97) in the PD-L1 CPS <10 subgroup (**Fig. 6A**), whereas the median OS was 19.6 months versus 16.9 months (HR, 0.83; 95% CI, 0.62–1.09) in the PD-L1 CPS ≥10 subgroup and 15.6 months versus 14.0 months (HR, 0.82; 95% CI, 0.65–1.03) in the PD-L1 CPS <10 subgroup (**Fig. 6B**).

When PFS and OS were evaluated by the prespecified cutoffs for both TMB and PD-L1 CPS, the HR of pembrolizumab versus chemotherapy and pembrolizumab plus chemotherapy versus chemotherapy were lower in the TMB ≥175 mutations/exome and PD-L1 CPS ≥10 dual biomarker subgroup than other subgroups defined by TMB and PD-L1 CPS cutoffs (Supplementary Figs. S3 and S4).

Discussion

This retrospective exploratory analysis in the first-line advanced urothelial carcinoma setting showed that TMB as a continuous

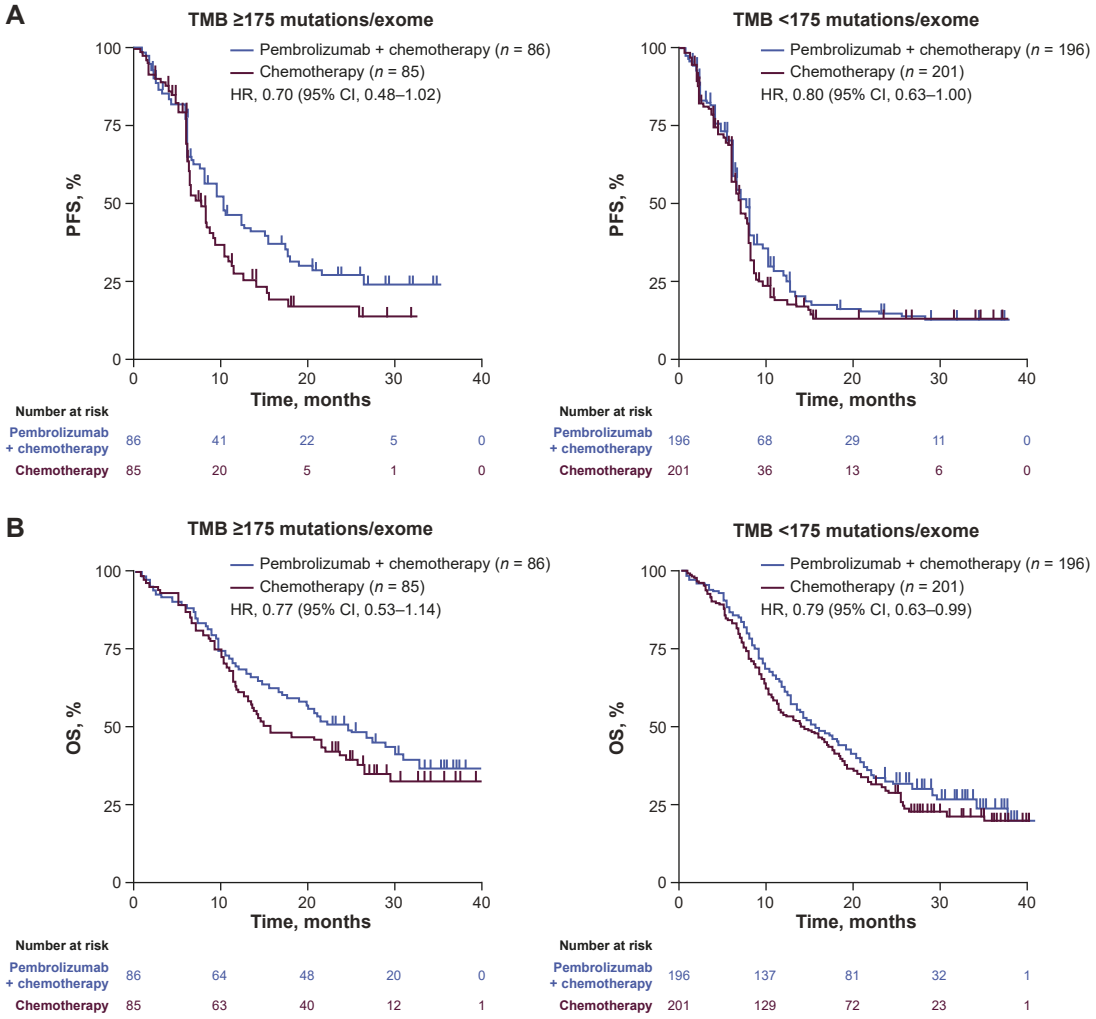

Figure 4.
Kaplan–Meier estimates of survival by TMB cutoff for pembrolizumab plus chemotherapy vs. chemotherapy. **A,** PFS. **B,** OS.

variable was associated with clinical outcomes (ORR, PFS, and OS) for pembrolizumab monotherapy, and a weaker association was observed for pembrolizumab plus chemotherapy (PFS and OS only) and chemotherapy alone (OS only). Pembrolizumab monotherapy or pembrolizumab plus chemotherapy was shown to have numerically longer median PFS and OS versus chemotherapy in the TMB ≥175 mutations/exome subgroup but not in the TMB <175 mutations/exome subgroup.

The observation that TMB is associated with clinical outcomes in this study is consistent with findings from studies of both first- and second-line anti–PD-(L)1 monotherapy in patients with advanced urothelial carcinoma (5, 9, 19) and across several indications (11). In the KEYNOTE-052 and KEYNOTE-045 studies of first- and second-line pembrolizumab monotherapy, respectively, TMB as a continuous variable was positively associated with ORR ($P < 0.001$ and $P = 0.007$, respectively), PFS ($P = 0.001$ and $P = 0.002$, respectively), and OS ($P = 0.012$ and $P = 0.015$, respectively) in patients with advanced urothelial carcinoma (9). The CheckMate 275 study of second-line nivolumab also showed that TMB was positively associated ($P < 0.05$) with ORR, PFS, and OS (20). In the current analysis of the KEYNOTE-361 study, the modest association between TMB as a continuous variable and OS in the chemotherapy-alone arm (two-sided $P = 0.040$) suggests that TMB is prognostic for OS in the advanced urothelial carcinoma setting; however, this result may be potentially confounded given that 61.1% of patients randomly assigned to the chemotherapy-alone arm received any subsequent therapy, including anti–PD-(L)1 therapy, after treatment discontinuation (15). In general, we have not observed consistent evidence to support the prognostic role of TMB for chemotherapy outcomes across several tumor types (9, 11, 21).

Trends observed when TMB was evaluated by a prespecified cutoff (175 mutations/exome equivalent to 10 mutations/megabase using FoundationOne CDx) are also consistent with those reported for patients with advanced urothelial carcinoma treated with an anti–PD-(L)1 therapy (5, 9, 19, 20, 22) and across several tumor types (11, 23). In the KEYNOTE-045 study, ORR was numerically higher with pembrolizumab versus chemotherapy in patients with TMB ≥175 mutations/exome tumors (35.2% vs. 15.1%), whereas a

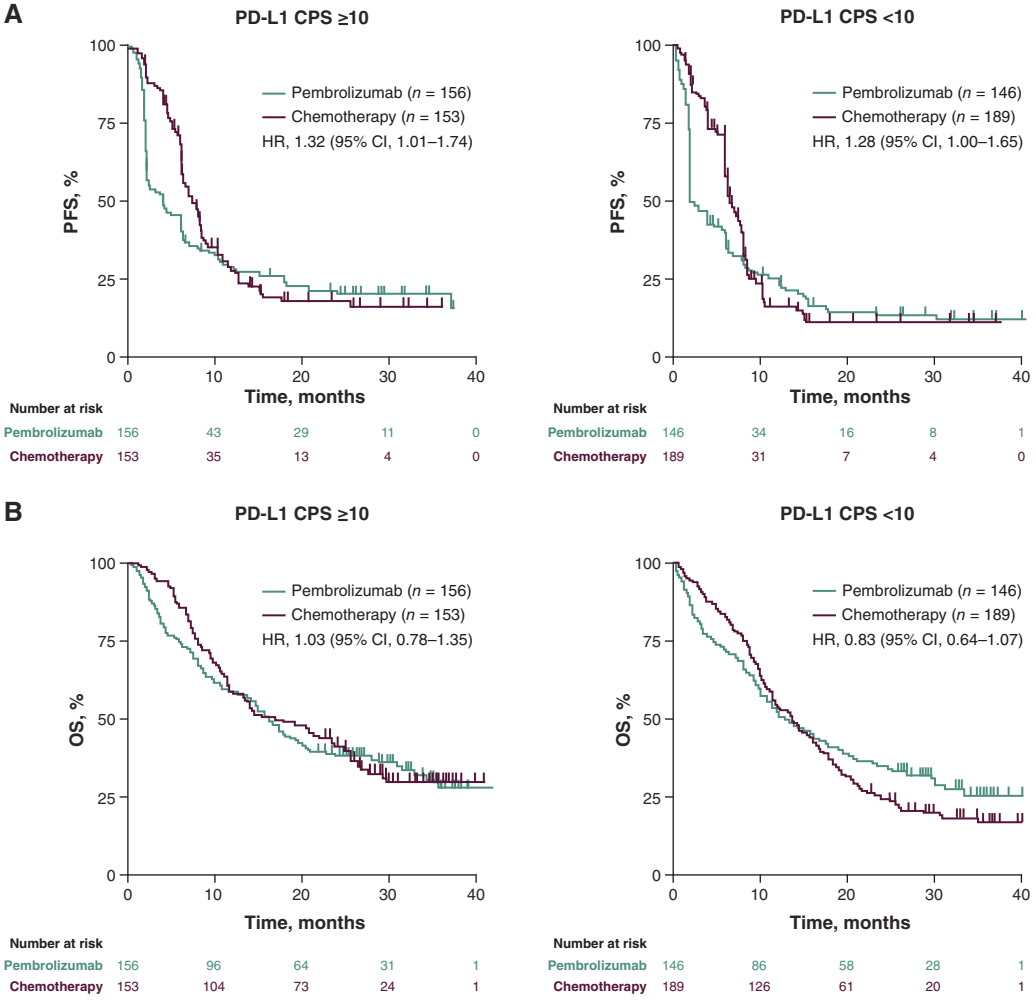

Figure 5.
Kaplan–Meier estimates of survival by PD-L1 CPS cutoff for pembrolizumab monotherapy vs. chemotherapy. **A,** PFS. **B,** OS.

subtle numerical difference in ORR was observed in patients with TMB <175 mutations/exome tumors (16.0% vs. 14.8%, respectively; ref. 9). In addition, a trend toward a lower OS HR for pembrolizumab versus chemotherapy was observed in patients with TMB ≥175 mutations/exome compared with TMB <175 mutations/exome (9). Similarly, in the phase III IMvigor130 study, a TMB cutoff of ≥10 mutations/megabase (equivalent to ≥175 mutations/exome via WES) was associated with longer OS with atezolizumab monotherapy versus chemotherapy (HR, 0.71; 95% CI, 0.49–1.03) and with atezolizumab plus chemotherapy versus chemotherapy (HR, 0.82; 95% CI, 0.58–1.17) in patients with advanced urothelial carcinoma (5). Similar trends for high TMB (by cutoff) and improved clinical outcomes have also been reported in phase II studies of first-line atezolizumab monotherapy (19) and second-line nivolumab monotherapy (20) in patients with advanced urothelial carcinoma. Although TMB has consistently been shown to predict response to anti–PD-(L)1 therapies in the advanced urothelial carcinoma setting (5, 9, 20, 24) and is also prognostic of survival outcomes in the pan-tumor setting independent of therapy (25), it is important to note that these results come from retrospective analyses and therefore need to be prospectively validated.

Testing of PD-L1 CPS as a continuous variable showed some weak trends toward positive association for both ORR and PFS, although statistical testing at the $\alpha = 0.05$ level did not show consistent associations with these outcomes for either pembrolizumab monotherapy or pembrolizumab plus chemotherapy, and in particular, no evidence of an association with OS was observed for pembrolizumab monotherapy. Furthermore, pembrolizumab monotherapy did not show trends for longer PFS and OS versus chemotherapy at CPS ≥10 or CPS <10. Such findings contrast with results from other studies evaluating PD-(L)1 inhibitor–based therapies in the first- or second-line setting in advanced or metastatic urothelial carcinoma (5, 10, 24, 26, 27). A meta-analysis of 1,436 patients with advanced urothelial carcinoma treated with either first-line or second-line anti–PD-(L)1 therapies across nine clinical studies showed that PD-L1 may serve as a predictive biomarker for ORR but not for OS (28).

Although different PD-L1 expression assays and cutoffs as well as patient populations are used across studies, the observation of

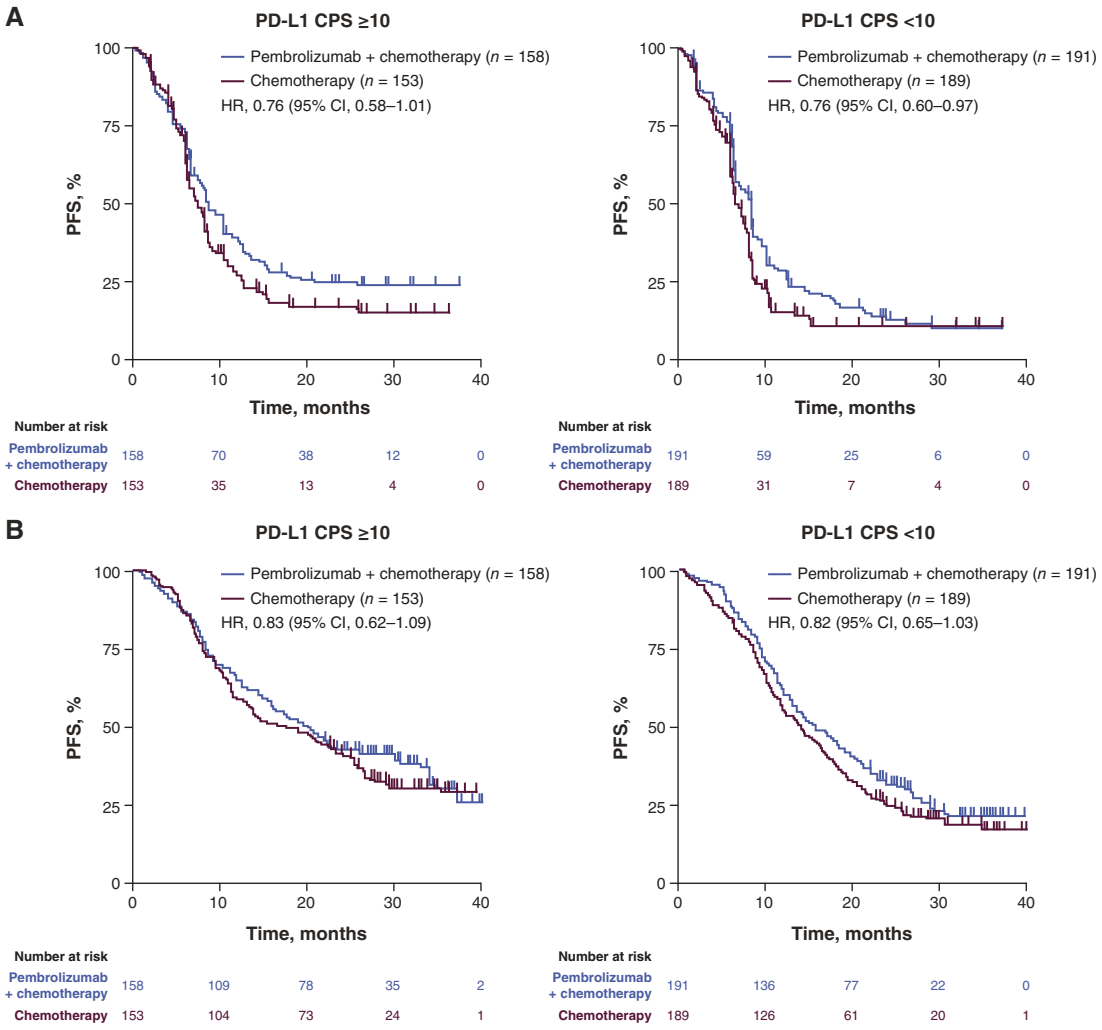

Figure 6.
Kaplan–Meier estimates of survival by PD-L1 CPS cutoff for pembrolizumab plus chemotherapy vs. chemotherapy. **A,** PFS. **B,** OS.

limited evidence of association between PD-L1 CPS and improved outcomes is somewhat consistent with the results from the phase II IMvigor210 (cohort 1) study, which showed that the response to first-line atezolizumab was not dependent on PD-L1 status in patients with advanced urothelial carcinoma (19). Results from a subgroup analysis of 131 Spanish patients pooled from the IMvigor210 (cohort 2) and IMvigor211 studies showed no significant differences in the efficacy of second-line atezolizumab (ORR, duration of response, PFS, and OS) regardless of PD-L1 expression status [immune cell (IC) 0/1 or IC 2/3; ref. 29]. Similarly, adjuvant atezolizumab monotherapy did not show treatment benefits based on PD-L1 status in the phase III IMvigor010 study of patients with high-risk muscle-invasive urothelial carcinoma (30).

The observed modest Spearman ρ for the correlation between TMB and PD-L1 CPS in all treatment arms suggests a positive but weak relationship between TMB and PD-L1 CPS as continuous variables in the first-line advanced urothelial carcinoma setting. Similar findings were reported for the correlation between TMB and PD-L1 CPS in patients with advanced urothelial carcinoma treated with pembrolizumab in the KEYNOTE-052 and KEYNOTE-045 studies (9). Our exploration of the joint effect of TMB and PD-L1 CPS on clinical outcomes showed evidence of improved ORR with high TMB (≥175 mutations/exome) and PD-L1 expression (CPS ≥10) for pembrolizumab monotherapy or pembrolizumab plus chemotherapy, as well as higher PFS and OS benefits for pembrolizumab monotherapy versus chemotherapy or pembrolizumab plus chemotherapy versus chemotherapy in the subgroup of patients with TMB ≥175 mutations/exome and PD-L1 CPS ≥10 tumors. These findings are consistent with a trend for improved clinical outcomes with higher TMB and PD-L1 CPS observed with first-line pembrolizumab monotherapy in the KEYNOTE-052 study (9) and in the results of the CheckMate 275 study, which showed that a combination of TMB and PD-L1 expression compared with either biomarker alone may better predict clinical outcomes with nivolumab treatment in patients with advanced urothelial carcinoma resistant to prior platinum-based chemotherapy (20). Similarly, the phase III IMvigor130 study showed that a

combination of high PD-L1 (IC 2/3) and high TMB (>10 mutations/megabase) favored OS with atezolizumab monotherapy versus placebo plus chemotherapy (HR, 0.22; 95% CI, 0.08–0.63) and with atezolizumab plus chemotherapy versus placebo plus chemotherapy (HR, 0.88; 95% CI, 0.48–1.62; ref. 5). Given the positive results with the antibody-drug conjugate enfortumab vedotin plus pembrolizumab (EV-302/KEYNOTE-A39; ref. 4) and nivolumab plus cisplatin-based chemotherapy (CheckMate 901 substudy; ref. 3) for patients with previously untreated advanced urothelial carcinoma, it is unknown if both TMB and PD-L1 expression could be jointly used to select patients who are likely to respond more effectively to these therapies.

Given that the number of patients with available TMB and PD-L1 CPS data represented 83% and 100%, respectively, of the treated population of the KEYNOTE-361 study, inferences drawn from the associations of these biomarkers and clinical outcomes are largely representative of the study population. Limitations of the current study include its exploratory nature and the unclear role of TMB for patient selection, particularly in light of recent data for enfortumab vedotin plus pembrolizumab from the phase III EV-302/KEYNOTE-A39 study and for nivolumab plus cisplatin-based chemotherapy from the phase III CheckMate 901 substudy that changed the standard-of-care treatment (3, 4). Additionally, the lack of information about histologic variants and pathologic stages is a limitation of this analysis given their importance to the treatment outcomes in patients with bladder cancer (31–35). Although TMB determined by WES differs slightly from TMB determined by FoundationOne CDx in other studies, a very high concordance between both methods has been shown in the pan-tumor setting (Spearman's correlation, 0.71), with 175 mutations/exome being the cutpoint that maximized the average positive and negative agreement with 10 mutations/megabase (AUROC, 0.92; ref. 11); thus, TMB results from this study are comparable with those reported in other studies.

In conclusion, this exploratory analysis from the randomized phase III KEYNOTE-361 study showed that TMB was associated with improved clinical outcomes for pembrolizumab monotherapy and, to a lesser extent, for pembrolizumab plus chemotherapy, in patients with advanced urothelial carcinoma. PD-L1 CPS was also associated with improved clinical outcomes for pembrolizumab monotherapy. In addition, in patients treated with either pembrolizumab alone or in combination with chemotherapy, improvement in outcomes over chemotherapy was highest in the TMB ≥175 mutations/exome and PD-L1 CPS ≥10 dual biomarker–defined subgroup. In light of new and emerging first-line immunotherapy-based treatments for advanced urothelial carcinoma, we continue to evaluate the role of these biomarkers in determining the response in this disease setting.

Authors' Disclosures

A. Fléchon reports personal fees and other support from MSD, Astellas Pharma, Merck & Co., Inc., Rahway, NJ, Bristol Myers Squibb, and Janssen and personal fees from Gilead outside the submitted work. R. Morales-Barrera reports serving in an advisory role for MSD, Pfizer, Merck & Co., Inc., Rahway, NJ, Janssen, and Astellas Pharma; received honoraria or travel expenses from Roche, Sanofi Aventis, Astellas, Janssen, MSD, Bayer, Merck & Co., Inc., Rahway, NJ, and Pfizer. T. Powles reports grants from AstraZeneca, Roche, Bristol Myers Squibb, Exelixis, Ipsen, MSD, Novartis, Pfizer, Seattle Genetics, Merck Serono, Astellas Pharma, Johnson & Johnson, and Eisai outside the submitted work. A. Alva reports grants from Merck & Co., Inc., Rahway, NJ, and V Foundation during the conduct of the study. Y. Loriot reports grants, personal fees, nonfinancial support, and other support from MSD during the conduct of the study, as well as personal fees, nonfinancial support, and other support from Janssen, Pfizer, Merck KGaA, Astellas Pharma, Gilead, Bristol Myers Squibb, and Roche; and other support from Incyte, Exelixis, and Taiho outside the submitted work. A. Rodriguez-Vida reports personal fees from Bristol Myers Squibb, Roche, Merck & Co., Inc., Rahway, NJ, Pfizer, Janssen, Astellas Pharma, Sanofi, Bayer, and AstraZeneca during the conduct of the study. S. Oudard reports personal fees from Sanofi, MSD, Ipsen, Novartis, Bayer, Janssen, and Astellas Pharma during the conduct of the study. C. Vulsteke reports grants from MSD and personal fees from MSD, Astellas Pharma, AstraZeneca, Bayer, Bristol Myers Squibb, GSK, Janssen-Cilag, LEO Pharma, Merck & Co., Inc., Rahway, NJ, and Pfizer outside the submitted work. R. Mamtani reports grants from Merck & Co., Inc., Rahway, NJ, and Astellas Pharma and personal fees from Seagen and Bristol Myers Squibb outside the submitted work. E.Y. Yu reports grants and personal fees from Merck & Co., Inc., Rahway, NJ, during the conduct of the study, as well as personal fees from Janssen, Advanced Accelerator Applications, Aadi Bioscience, Bristol Myers Squibb, and Loxo; grants and personal fees from Bayer, Oncternal, and Lantheus; and grants from Dendreon, Seagen, Blue Earth, Tyra, and Daiichi Sankyo outside the submitted work. A. Montesa Pino reports personal fees from Bristol Myers Squibb, Astellas Pharma, AstraZeneca, Novartis, MSD, Janssen, Bayer, and Advanced Accelerator Applications and personal fees and nonfinancial support from Pfizer, Merck & Co., Inc., Rahway, NJ, and Ipsen outside the submitted work. U. Anido reports personal fees and nonfinancial support from Ipsen; personal fees from Bristol Myers Squibb, Eisai, MSD, and Merck & Co., Inc., Rahway, NJ; and grants and personal fees from Bayer outside the submitted work. O. Huillard reports personal fees from Advanced Accelerator Applications, Bayer, Sanofi, MSD, Bristol Myers Squibb, Ipsen, Pfizer, Eisai, AstraZeneca, Novartis, and Janssen outside the submitted work. J. Ma is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ. M. Rajasagi was an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, at the time of this analysis. A. Vajdi is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, and owns stock in Merck & Co., Inc., Rahway, NJ. J. Lunceford is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, and owns stock in Merck & Co., Inc., Rahway, NJ. R. Cristescu is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, and owns stock in Merck & Co., Inc., Rahway, NJ. K. Imai is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, and owns stock in Merck & Co., Inc., Rahway, NJ. B. Homet Moreno is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, and owns stock in Merck & Co., Inc., Rahway, NJ. N. Matsubara reports grants from MSD during the conduct of the study, as well as personal fees from Sanofi and grants from AbbVie, Amgen, Astellas Pharma, AstraZeneca, Boehringer Ingelheim, Chugai Pharma, Eisai, Janssen, Eli Lilly and Company, Loxo Oncology, Novartis, Bayer, Pfizer, Roche, Seagen, Taiho, and Takeda outside the submitted work. No disclosures were reported by the other authors.

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Note

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